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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/528,673	03/23/2005	Tatsuo Hoshino	K21409USWO C038435/018565	2412
7590	02/01/2008	Stephen M Haracz Bryan Cave 1290 Avenue of the Americas New York, NY 10104	EXAMINER RAGHU, GANAPATHIRAM	
			ART UNIT 1652	PAPER NUMBER
			MAIL DATE 02/01/2008	DELIVERY MODE PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/528,673	HOSHINO ET AL.	
	Examiner	Art Unit	
	Ganapathirama Raghu	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) Responsive to communication(s) filed on 15 November 2007.
- 2a) This action is FINAL. 2b) This action is non-final.
- 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) Claim(s) 1-4 and 6-18 is/are pending in the application.
- 4a) Of the above claim(s) 3,4,9-12,14,15,17 and 18 is/are withdrawn from consideration.
- 5) Claim(s) _____ is/are allowed.
- 6) Claim(s) 1, 2, 6-8, 13 and 16 is/are rejected.
- 7) Claim(s) _____ is/are objected to.
- 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) The specification is objected to by the Examiner.
- 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date: _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date: _____ | 6) <input type="checkbox"/> Other: _____ |

Application Status

In response to the Office Action mailed on 05/11/2007, applicants' filed a response and amendment received on 11/15/2007. Said amendment, amended claims 1, 2 and 8 and cancelled claim 5. Thus, Claims 1-4 and 6-18 are pending in the application. Claims 3, 4, 9-12, 14-15 and 18 remain withdrawn as they are drawn to non-elected inventions. Claims 1, 2, 6-8, 13 and 16 are now under consideration for examination.

New Matter-Claim Rejections 35 USC § 112

Claims 1, 2, 8 and claims 6, 7, 13 and 16 depending therefrom are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 1, 2, 8 are rejected because the phrase "directly" is new matter. The scope of the process for the production of L-ascorbic acid comprising: (a) contacting an enzyme with a substrate which is selected from the group consisting of L-gulose, L-galactose, L-iodose and L-talose; (b) converting the substrate "directly" into L-ascorbic acid as claimed was not contemplated in the specification as originally filed. The process for the production of L-ascorbic acid comprising: (a) contacting an enzyme with a substrate which is selected from the group consisting of L-gulose, L-galactose, L-iodose and L-talose; (b) converting the substrate into L-ascorbic acid as in original claims 1, 2, 8 has a different scope than the process now claimed.

Applicants' claim to have support for this amendment in the specification at for example, page 1, lines 1-2; page 2, lines 8-12; page 2, line 25 to page 3, line 9 and lines 27-29; in

examples 1-4 and Tables 1-4 and in original claims 1 and 5 without addressing the issues raised by the examiner in the Office action dated 05/11/2007.

Reply: In the sections pointed out by the applicant for the support, examiner is unable to find either explicit or implicit meaning wherein the claims or the specification construes/contemplates converting the substrates directly into L-ascorbic acid by catalytic activity of a polypeptide having the amino acid sequence of SEQ ID NO: 2. Examiner has thoroughly explained his position in the previous Office Action (05/11/2007) regarding interpretation of claims and support in the specification and therefore continues to hold the position that: 1) The claims as written “A process for production of L-ascorbic acid comprising: is interpreted as “open language” and therefore the process for production of L-ascorbic acid can comprise other elements in the reaction. 2) Furthermore, neither the claims as written nor the specification explicitly states that the said process for the production of L-ascorbic acid is a direct one step-conversion of claimed substrates into L-ascorbic acid as amended. The said process of production of L-ascorbic acid was carried out under specific cellular context in vivo, i.e., production of L-ascorbic acid in a process comprising: contacting an enzyme having the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide of SEQ ID NO: 1, said polypeptide expressed in a specific strain of *E.coli* JM 109 having the activity to produce L-ascorbic acid from substrates L-gulono-1,4-lactone/L-gulonic acid from L-gulose and from L-galactono-1,4-lactone/L-galctonic acid or conversion of substrate L-galactose to L-galactono-1,4-lactone/L-galactonic acid and L-ascorbic acid under suitable culture conditions (as in Examples: 1-4 and Tables 1-4, pages 8-10; and culture conditions: lines 15-28, page 6 of specification) and therefore said bacteria may provide other necessary enzymes either for the

production of intermediate products of L-ascorbic with claimed substrates or for the final conversion of the intermediate products to L-ascorbic acid. Therefore said process of production of L-ascorbic acid does not involve converting substrates directly into L-ascorbic acid.

Furthermore claimed recitation of a use of Enzyme B ... (in page 1, lines 1-2 of specification), without setting forth any steps involved in the process, results in an improper definition of a process (See for example *Ex parte Dunki*, 153 USPQ 678 (Bd.App. 1967) and *Clinical Products, Ltd. v. Brenner*, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966). The recitation of phrase "use" without any active, positive steps delimiting how this use is actually practiced, renders the term indefinite and failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

New-Claim Rejections: 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention..

Claims 1, 2 and claims 6, 7 and 13 dependent therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1 and 2 recites the phrase "...under stringent hybridization and wash conditions...", the metes and bounds of this claim are not clear to the examiner. It is well known in the art that stringent hybridization and wash conditions can be described as high stringency, medium stringency or low stringency. It is not clear to the examiner as to what type of stringency is encompassed in the above phrase. While page 4 of the specification describes some conditions which are intended to be stringent, there

is nothing to suggest that other conditions would not also be included within the scope of this term and in the art what is considered stringent varies widely depending on the individual situation as well as the person making the determination. Clarification and Correction is required.

Maintained- Claim Rejections: 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-2, 6-8, 13 and 16 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the production of L-ascorbic acid comprising: contacting an enzyme having the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide of SEQ ID NO: 1, said polypeptide expressed in a specific strain of *E.coli* JM 109 having the activity to produce L-ascorbic acid from substrates L-gulono-1,4-lactone/L-gulonic acid from L-gulose and from L-galactono-1,4-lactone/L-galctonic acid or conversion of substrate L-galctose to L-galactono-1,4-lactone/L-galactonic acid and L-ascorbic acid under suitable culture conditions (as in Examples: 1-4, pages 8-10; and culture conditions: lines 15-28, page 6 of specification). However, the specification does not reasonably provide enablement for a process for the production of L-ascorbic acid comprising: contacting an enzyme with a substrate selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid and converting the substrate directly into L-ascorbic acid by catalytic activity of the enzyme under suitable culture conditions, wherein said enzyme has an

amino acid sequence 90% identical to SEQ ID NO: 2 from any source including variants, mutants and recombinants and encoded by the polynucleotide of SEQ ID NO: 1 or an amino acid sequence encoded by a polynucleotide that hybridizes to SEQ ID NO: 1 under undefined stringent hybridization and wash conditions and further said polypeptide expressed in any cellular context is able to produce L-ascorbic acid in a process for the production of L-ascorbic acid under specific defined process conditions such as pH, temperature and time in which said substrates are allowed to react with said enzyme. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention commensurate in scope with the claims.

Previous Reply in the Office Action of letter dated 05/11/2007.

Applicants' have traversed this rejection and the claimed invention is enabled if any person skilled in the art can make and use the invention without undue experimentation and need only routine experimentation as the burden lies with the examiner to provide reasons for the uncertainty of the enablement. Applicants' arguments have been considered and the following body of scientific publication supports the basis for rejection.

While methods to produce variants of a known sequence, such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan, producing variants capable of being used in a process for the production of L-ascorbic acid comprising: contacting an enzyme with a substrate selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid, wherein said enzyme

has an amino acid sequence 90% identical to SEQ ID NO: 2 from any source including variants, mutants and recombinants and encoded by the polynucleotide of SEQ ID NO: 1 or an amino acid encoded by a polynucleotide that hybridizes to SEQ ID NO: 1 under stringent hybridization conditions and said polypeptide under any conditions i. e., said polypeptide expressed in any cellular context is able to produce L-ascorbic acid in a process for the production of L-ascorbic acid under specific defined process conditions such as pH, temperature and time in which any substrate i. e., selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid are allowed to react with said enzyme, requires that one of ordinary skill in the art know or be provided with guidance for the selection of which, of the infinite number of variants, have the activity. Without such guidance, one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. For the rejected claims, this would clearly constitute **undue** experimentation. Guo et al., (PNAS, 2004, Vol. 101 (25): 9205-9210) teach that the percentage of random single-substitution mutations, which inactivate a protein, using a protein 3-methyladenine DNA glycosylase as a model, is 34% and that this number is consistent with other studies in other proteins (p 9206, paragraph 4). Guo et al., (*supra*) further show that the percentage of active mutants for multiple mutations appears to be exponentially related to this by the simple formula $(.66)^x \times 100\%$ where x is the number of mutations introduced (Table 1). Applying this estimate to the protein recited in the instant application, 90% identity allows up to 58 mutations within the 579 amino acids of SEQ ID NO: 2 and, thus, only $(0.66)^{58} \times 100\%$ or $3.44 \times 10^{-9}\%$ of random mutants having 90% identity would be active. Current techniques in the

art (i.e., high throughput mutagenesis and screening techniques) would allow for finding a reasonable number of active mutants within hundred thousand inactive mutants (despite even this being an enormous quantity of experimentation that would take a very long time to accomplish). But finding a few mutants within several billions or more, as in the claims to 90% identity, would not be possible. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification.

Applying this estimate to the instant protein, a functional equivalent thereof with 90% sequence identity, as recited in Claims 1-2, 6-8, 13 and 16, an extremely low number of active mutants will be present among an enormously large number of inactive mutants and as such screening for these active mutants would be burdensome and undue experimentation when there is no guidance provided in the specification.

Applicants continue to argue that:

- (A) The rejection, fails to identify or articulate any factual basis or supporting evidence to establish that undue experimentation is required to practice the invention.
- (B) We further note that independent claims 1, 2 and 8 have been amended to explicitly recite a direct one-step conversion of substrate into L-ascorbic acid.
- (C) Specification discloses that a “functional equivalent... DNA sequences which hybridize under standard conditions with such sequences or fragments thereof...”

Reply: (A) The examiner in the previous reply (Office Action of letter dated 05/11/2007) has clearly articulated and presented a factual basis with supporting scientific evidence; Guo et

al., (PNAS, 2004, Vol. 101 (25): 9205-9210) why a skilled artisan requires specific guidance regarding making mutants as claimed in the instant claims. Claims are interpreted in the light of the specification, however, specification cannot be read into the claims, therefore when claims are given the broadest interpretation, the claims read on random mutants simply having 90% sequence identity to SEQ ID NO: 2 and therefore a functional equivalent thereof with 90% sequence identity, as recited in Claims 1-2, 6-8, 13 and 16, an extremely low number of active mutants will be present among an enormously large number of inactive mutants and as such screening for these active mutants would be burdensome and undue experimentation when there is no guidance provided in the specification.

(B) Claims 1, 2, 8 and claims 6, 7, 13 and 16 depending therefrom are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 1, 2, 8 are rejected because the phrase "directly" is new matter.

(C) Claims 1 and 2 recites the phrase "...under stringent hybridization and wash conditions...", the metes and bounds of this claim are not clear to the examiner.

Withdrawn-Claim Rejections 35 USC § 102

Previous rejection of claims 1-2, 6-8, 13 and 16 under 35 U.S.C. 102(b) as being anticipated by Asakura et al., (EPO 832974 A2, date of publication 01/04/1998) is being withdrawn. Amendment to claims has necessitated the withdrawal of the instant rejection. However, examiner for the record would like to state that if the "new-matter" is cancelled by the

applicants, the rejection will be reinstated and such an action by the examiner will not be considered as new ground of rejection.

Withdrawn- Claim Rejections 35 USC § 103

Previous rejection of claims 1, 2, 6-8, 13 and 16 under 35 U.S.C. 103(a) as being unpatentable over Asakura et al., (EPO 832974 A2, date of publication 01/04/1998) and further in view of Bourdant et al., (Enzyme Micro. Technol., 1990, Vol. 12, pages 322-329) and Hancock et al., (TRENDS in Biotechnol., 2002, Vol. 20 No. 7, pages 299-305) is being withdrawn. Amendment to claims has necessitated the withdrawal of the instant rejection. However, examiner for the record would like to state that if the “new-matter” is cancelled by the applicants, the rejection will be reinstated and such an action by the examiner will not be considered as new ground of rejection.

Maintained-Claim Rejections 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 2, 8, 13 and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by Sugisawa et al., (1995) when given the broadest interpretation. Claims 2, 8, 13 and 16 are directed to a process for the production of L-ascorbic acid comprising: contacting an enzyme directly with a substrate selected from the group consisting of, L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid, with an enzyme

derivable from of *G.oxydans* DSM 4025 and converting the said substrates into L-ascorbic acid by catalytic activity of the enzyme under suitable culture conditions and isolating L-ascorbic acid from the reaction, wherein said enzyme has the following physico-chemical properties: a) molecular weight of about 60, 000 Da on SDS-PAGE; b) substrate specificity for primary and secondary alcohols and aldehydes; c) pH stability at a pH of about 6 to about 9; d) pH optimum of about 8.0; and e) inhibited by Cu²⁺, Zn²⁺, Mn²⁺, Fe²⁺ and Fe³⁺ under specific defined process conditions such as pH, temperature and time in which the substrates are allowed to react with said enzyme.

Sugisawa et al., (*supra*) disclose the purification, kinetic profiles and physico-chemical characterization of a polypeptide derived from *G.oxydans* DSM 4025 that produced L-ascorbic acid from L-gulono-γ-lactone said enzyme consisted of 3 subunits of molecular weight of about 61,000 +/- 1000, 32,500 +/- 1000 and 16,500 +/- 500 with identical physico-chemical properties and substrate specificity, optimal pH range, pH stability, thermal stability and effect of metals and inhibitors on the activity of said enzyme (Abstract section). Furthermore, Tables: I, II, IV, V, VI and VII disclose production of L-ascorbic acid, substrate specificity, effects of temperature, pH and various metals on the activity of said enzyme. Claims 2, 13 and 16 are included in the rejection although said claims recite specific SEQ ID NO: 2 and the activity of said polypeptides under specific pH and temperature, because examiner interprets these properties to be inherent in the isolated polypeptide. Therefore the reference of Sugisawa et al., anticipates the claims 2, 8, 13 and 16 as written.

Since the Office does not have the facilities for examining and comparing applicants' protein with the protein of the prior art, the burden is on the applicant to show a novel or

unobvious difference between the claimed product and the product of the prior art (i.e., that the protein of the prior art does not possess the same material structural and functional characteristics of the claimed protein). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

Applicants' have traversed the rejection with the argument that "the independent claims 2 and 8 have been amended to explicitly recite a direct one-step conversion of substrate into L-ascorbic acid".

Reply: Sugisawa et al., (*supra*) disclose the purification, kinetic profiles and physico-chemical characterization of a polypeptide derived from *G.oxydans* DSM 4025 that produced L-ascorbic acid from L-gulono- γ -lactone one of the substrates utilized for the production and as claimed in instant claims (see Results section, pages 191-192, especially section 3 of Results, page 192).

Summary of Pending Issues

The following is a summary of issues pending in the instant application.

1. Amended claims 1, 2, 8 and claims 6, 7, 13 and 16 depending therefrom are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement for New-matter.
2. Amended claims 1, 2 and claims 6, 7 and 13 dependent therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. Previous rejections of claims 1-2, 6-8, 13 and 16 rejected under 35 U.S.C. 112, first paragraph, for enablement is maintained.
4. Previous rejections of claims 2, 8, 13 and 16 rejected under 35 U.S.C. 102(b) as being anticipated by Sugisawa et al., (1995) when given the broadest interpretation is maintained.

Allowable Subject Matter/Conclusion

None of the claims are allowable.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate pages.

It is also requested that Applicants identify support, within the original application, for any amendments to the claims and specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathirama Raghu whose telephone number is 571-272-4533. The examiner can normally be reached on M-F; 8:00-4:30 pm EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of the application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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